植物研究雑誌
J. Jpn. Bot.
71: 333–337 (1996)

A Comparative Chromosome Study of *Rubus* × *nikaii*, *R. parvifolius* and *R. phoenicolasius* (Rosaceae)

Yoshikane IWATSUBO and Naohiro NARUHASHI

Department of Biology, Faculty of Science, Toyama University,
Gofuku, Toyama, 930 JAPAN

(Received on June 24, 1996)

Rubus × *nikaii* was originally described by Ohwi (1949) who considered it as a spontaneous hybrid between *R. parvifolius* and *R. phoenicolasius*. In order to provide more conclusive evidence for the putative origin of this taxon, karyotypes and meiotic chromosome behavior of *R.* × *nikaii*, *R. parvifolius* and *R. phoenicolasius* were investigated. These karyotype studies support the taxonomic interpretation of Ohwi (1949). The karyotypic similarity among the three taxa and the most frequent kind of chromosome pairing in PMCs of *R.* × *nikaii*, 7II, suggests that *R. parvifolius* and *R. phoenicolasius* are closely related species with similar genomes.

Rubus × *nikaii* Ohwi, collected for the first time by Nikai at Okotsu, Ohda-mura, Yamaguchi Prefecture, Japan in 1919, was originally described by Ohwi (1949), who considered this plant to be a spontaneous hybrid between *R. parvifolius* Linn. and *R. phoenicolasius* Maxim. This taxon is known to be diploid with 2n=14 (Iwatsubo and Naruhashi 1993a), which is in accordance with the ploidy level of both its putative parents, *R. parvifolius* (Jinno 1958a, 1958b, Iwatsubo and Naruhashi 1991, 1993b) and *R. phoenicolasius* (Longley and Darrow 1924, Jinno 1958a, 1958b, Iwatsubo and Naruhashi 1993b). This study was undertaken to provide more conclusive evidence for the postulated origin of this taxon by an analysis of karyotypes and meiotic chromosome behavior of *R.* × *nikaii*, *R. parvifolius* and *R. phoenicolasius*.

Materials and Methods

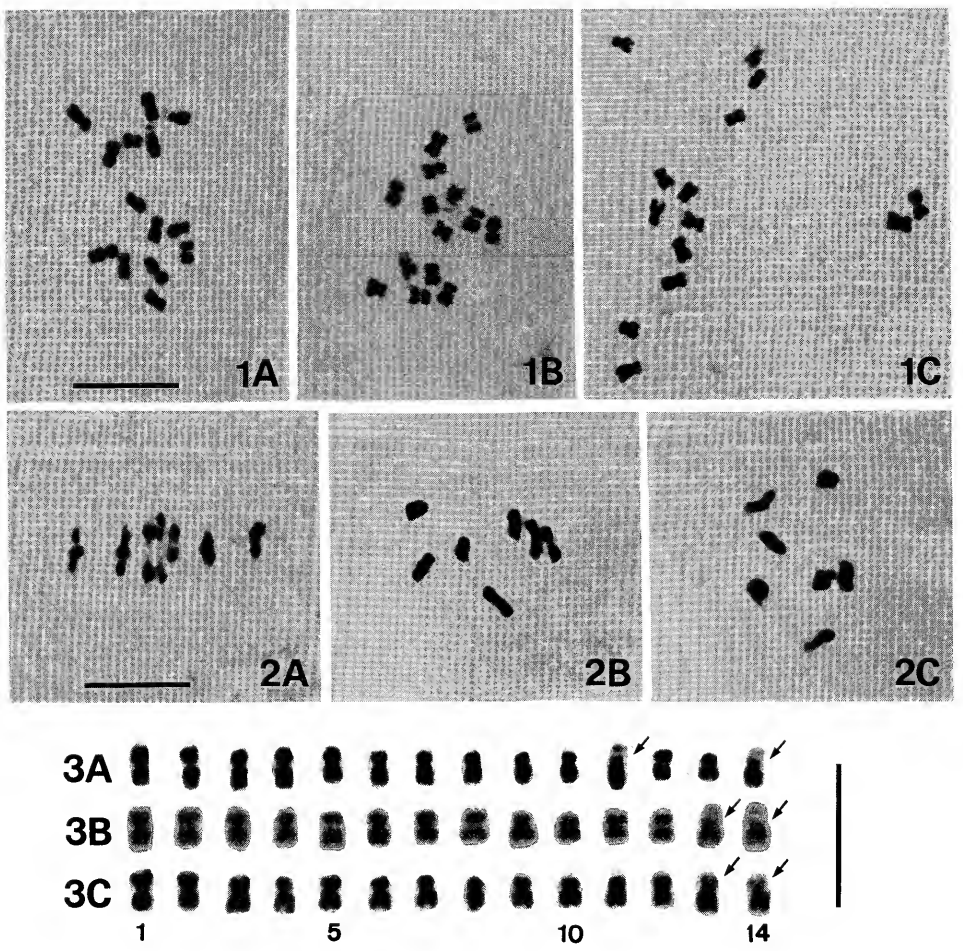
One plant each of *R.* × *nikaii*, *R. parvifolius* and *R. phoenicolasius* was used for the study. Plants were cultivated in the botanical garden of Toyama University. *Rubus* × *nikaii* and *R. phoenicolasius* were the same individuals for which chromosome numbers were previously determined (Iwatsubo and Naruhashi 1991, 1993b). Their original collection localities are: *R.* × *nikaii*: Yokota-cho, Nitta-gun, Shimane Prefecture; *R. parvifolius*: Toyama-shi, Toyama Prefecture; and *R. phoenicolasius*: Ogi-machi, Sado-gun, Niigata Prefecture.

Karyotypic analyses were made from preparations of rapidly growing root tips. Root tips collected from potted plants were pretreated in a 2mM 8-hydroxyquinline solution at room temperature for one hour and then at ca. 5°C for 15 hours. Fixation was carried out in a fresh mixture of glacial acetic acid and absolute ethyl alcohol (1:3) for one hour. Then root

tips were soaked in 1N hydrochloric acid for a few hours, followed by maceration in 1N hydrochloric acid at 60°C for 11.5 minutes. Then, root tips were immersed in tap water for more than 3 minutes, before being stained and squashed in 1.5% lacto-propionic orcein. Cells having well scattered metaphase chromosomes were used for the karyotypic studies. Chromosome forms were expressed following the nomenclature recommended by Levan et al. (1964).

Meiotic chromosome studies were carried out in pollen mother cells (PMCs). Young flower buds were fixed in Newcomer's fluid at 17°C for three hours and macerated with the same procedure as for the root tips. After being stained with Schiff's reagent, anthers were stained and squashed in 1.5% lacto-propionic orcein, and chromosome pairing at first meiotic metaphase (MI) of PMCs was examined.

Pollen fertility of *R.* ×*nikaii* was estimated on the basis of its size and stainability in 1.5% lacto-propionic orcein in more than two thousand pollen grains.



Figs. 1–3. Somatic chromosomes at metaphase (Fig. 1) in root tips, meiotic chromosomes at metaphase I (Fig. 2) in pollen mother cells, and karyotypes (Fig. 3) of *Rubus* ×*nikaii*, *R. parvifolius* and *R. phoenicolasius*. 1A, 2A & 3A: *R.* ×*nikaii*, 2n=14, 7II; 1B, 2B and 3B: *R. parvifolius*, 2n=14, 7II; 1C, 2C and 3C: *R. phoenicolasius*, 2n=14, 7II. Arrows indicate satellite chromosomes. Bars represent 7 μm.

Results and Discussion

Descriptions of the somatic chromosome complements and meiotic chromosome pairing in the three plants are as follows:

1) *Rubus* × *nikaii* (2n=14, Figs. 1A, 3A)

The chromosomes at somatic metaphase ranged from 1.0 μm to 1.6 μm in length and 1.0 to 5.0 in arm ratio (Table 1). This somatic chromosome complement consisted of ten metacentric chromosomes, two submetacentric chromosomes, and two subtelocentric chromosomes. The two subtelocentric ones had satellites on the short arms and were different from each other in both total length and arm ratio. Chromosome pairing was examined in 208 PMCs at MI. Most cells showed seven bivalents (Fig. 2A, Table 4). Pollen fertility of this plant was 2.8%, and the plant set no seed.

2) *Rubus parvifolius* (2n=14, Figs. 1B, 3B)

The length of the somatic metaphase chromosomes ranged from 0.9 μm to 1.4 μm, and the arm ratio varied from 1.0 to 3.5 (Table 2). The somatic chromosome complement was composed of ten metacentric chromosomes, two submetacentric chromosomes and two subtelocentric chromosomes. Satellites were found on the short arms of both the subtelocentric ones. Chromosome pairing was examined in 53 PMCs at MI. All cells had seven bivalents (Fig. 2B, Table 4).

3) *Rubus phoenicolasius* (2n=14, Figs. 1C, 3C)

The somatic metaphase chromosomes ranged from 1.2 μm to 1.6 μm in length and 1.0 to 5.0 in arm ratio (Table 3). They were classified into three groups: ten metacentric chromosomes, two submetacentric chromosomes, and two subtelocentric chromosomes. The subtelocentric ones had satellites on their short arms. Chromosome pairing at MI was examined in 150 PMCs. All of them showed seven bivalents (Fig. 2C, Table 4).

Karyotypes of the three taxa are uniformly formulated as 2n=14=10m+2sm+2^{t}st. However, there were differences in the subtelocentric chromosomes among

Table 1. Measurements at somatic metaphase chromosomes of *Rubus* × *nikaii*

No.	Length	Total (μm)	A.R.	Form	No.	Length	Total (μm)	A.R.	Form
1	0.8 + 0.8	1.6	1.0	M	8	0.5 + 0.8	1.3	1.6	m
2	0.7 + 0.9	1.6	1.3	m	9	0.5 + 0.7	1.2	1.4	m
3	0.6 + 1.0	1.6	1.7	m	10	0.5 + 0.7	1.2	1.4	m
4	0.6 + 1.0	1.6	1.7	m	11	t–0.2 + 1.0	1.2	5.0	st
5	0.7 + 0.8	1.5	1.1	m	12	0.5 + 0.6	1.1	1.2	m
6	0.5 + 0.9	1.4	1.8	sm	13	0.5 + 0.5	1.0	1.0	M
7	0.5 + 0.9	1.4	1.8	sm	14	t–0.2 + 0.8	1.0	4.0	st

t: satellite

Table 2. Measurements at somatic metaphase chromosomes of *Rubus parvifolius*

No.	Length	Total (μm)	A.R.	Form	No.	Length	Total (μm)	A.R.	Form
1	0.7 + 0.7	1.4	1.0	M	8	0.5 + 0.5	1.0	1.0	M
2	0.7 + 0.7	1.4	1.0	M	9	0.4 + 0.6	1.0	1.5	m
3	0.6 + 0.8	1.4	1.3	m	10	0.4 + 0.6	1.0	1.5	m
4	0.6 + 0.7	1.3	1.2	m	11	0.4 + 0.5	0.9	1.3	m
5	0.4 + 0.8	1.2	2.0	sm	12	0.4 + 0.5	0.9	1.3	m
6	0.4 + 0.8	1.2	2.0	sm	13	t–0.2 + 0.7	0.9	3.5	st
7	0.5 + 0.6	1.1	1.2	m	14	t–0.2 + 0.7	0.9	3.5	st

t: satellite

Table 3. Measurements at somatic metaphase chromosomes of *Rubus phoenicolasius*

No.	Length	Total (μm)	A.R.	Form	No.	Length	Total (μm)	A.R.	Form
1	0.8 + 0.8	1.6	1.0	M	8	0.5 + 0.8	1.3	1.6	m
2	0.8 + 0.8	1.6	1.0	M	9	0.6 + 0.6	1.2	1.0	M
3	0.6 + 1.0	1.6	1.7	m	10	0.6 + 0.6	1.2	1.0	M
4	0.6 + 1.0	1.6	1.7	m	11	0.5 + 0.7	1.2	1.4	m
5	0.5 + 0.9	1.4	1.8	sm	12	0.5 + 0.7	1.2	1.4	m
6	0.5 + 0.9	1.4	1.8	sm	13	t–0.2 + 1.0	1.2	5.0	st
7	0.5 + 0.8	1.3	1.6	m	14	t–0.2 + 1.0	1.2	5.0	st

t: satellite

the three taxa: (1) The arm ratio of both chromosomes of *R. parvifolius* was 3.5, and that of both chromosomes of *R. phoenicolasius* was 5.0, while that of the two chromosomes in *R.* × *nikaii* was 4.0 and 5.0, respectively; (2) The length of both subtelocentric chromosomes of *R. parvifolius* was 0.9 μm, and that of both chromosomes of *R. phoenicolasius* was 1.2 μm, while that of the two chromosomes in *R.* × *nikaii* was 1.0 μm and 1.2 μm, respectively. The smaller subtelocentric chromosome of *R.* × *nikaii* is similar in length and in arm ratio to the subtelocentric chromosomes of *R. parvifolius*, while the longer one is similar to those parameters of *R. phoenicolasius*. As a rule, the karyotype of a hybrid will show a chromosome complement representing one set from each parent plant. Thus the hybrid between *R. parvifolius* and *R. phoenicolasius* should have a somatic chromosome complement formulated as 2n=14=10m+2sm+2^{t}st and, as expected, its two satellite chromosomes differ in arm ratio. The longer satellite chromosome with a higher arm ratio is thought to be derived from *R. phoenicolasius*, while the shorter one with a lower arm ratio is derived from *R. parvifolius*. *Rubus* × *nikaii* is, therefore, regarded as having originated as a hybrid, coinciding with the taxonomic interpretation of Ohwi (1949). This plant never set fruit and its pollen was almost all abortive, further evidence for a hybrid origin.

Rubus parvifolius and *R. phoenicolasius* are both placed in the section Parvifolii of the subgenus Idaeobatus (Naruhashi 1980). Their karyotypic similarity and the regular bivalent pairing in PMCs of *R.* × *nikaii* suggests that *R. parvifolius* and *R. phoenicolasius* are closely related species with similar genomes.

Table 4. Chromosome pairing at first metaphase in PMCs of *Rubus* × *nikaii*, *R. parvifolius* and *R. phoenicolasius*

Taxa Chromosome pairing	No. of PMCs	Frequency (%)
R. × *nikaii* (2n=14)		
7II	205	98.6
6II + 2I	3	1.4
R. parvifolius (2n=14)		
7II	53	100.0
R. phoenicolasius (2n=14)		
7II	150	100.0

The authors are grateful to Dr. Maxine Thompson for her critical reading of the manuscript.

References

Iwatsubo Y. and Naruhashi N. 1991. Karyomorphological and cytogenetical studies of *Rubus parvifolius*, *R. coreanus* and *R.*×*hiraseanus* (Rosaceae). Cytologia **56**: 151–156.

——— and ——— 1992. Cytotaxonomical studies of *Rubus* (Rosaceae) I. Chromosome numbers of 20 species and 2 natural hybrids. J. Jpn. Bot. **67**: 270–275.

——— and ——— 1993a. Cytotaxonomical studies of *Rubus* (Rosaceae) II. Chromosome numbers of 21 species and 6 natural hybrids. J. Jpn. Bot. **68**: 159–165.

——— and ——— 1993b. Cytogenetical study of *Rubus* × *tawadanus* (Rosaceae). Cytologia **58**: 217–221.

Jinno T. 1958a. Cytogenetic and cytoecological studies on some Japanese species of *Rubus* I. Chromosomes. Bot. Mag. Tokyo **71**: 15–22.

——— 1958b. Cytogenetic and cytoecological studies on some Japanese species of *Rubus* II. Cytogenetic studies on some F_1-hybrid. Jap. J. Genet. **33**: 201–209.

Levan A., Fredga K. and Sandberg A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.

Longley A. E. and Darrow G. M. 1924. Cytological studies of diploid and polyploid forms in raspberries. J. Agric. Res. **27**: 737–748.

Naruhashi N. 1980. Morphology of 34 Japanese *Rubus* species. Acta Horticulturae **112**: 177–181.

Ohwi J. 1949. Notes on some plants from the Far East. Bull. Nat. Sci. Mus. **26**: 1–12.

岩坪美兼，鳴橋直弘: **キイチゴ属アイノコキイチゴ，ナワシロイチゴ，エビガライチゴの染色体比較**

アイノコキイチゴ *Rubus* × *nikaii* Ohwi は，ナワシロイチゴ *R. parvifolius* Linn. とエビガライチゴ *R. phoenicolasius* Maxim. の自然雑種であるとみなされている（Ohwi 1949）．これら3分類群の核型の比較と，それぞれの花粉母細胞染色体の観察をおこなった．その結果，ナワシロイチゴとエビガライチゴの間ではサテライト染色体に形態的違いが認められた．またアイノコキイチゴの2本のサテライト染色体は，それぞれナワシロイチゴとエビガライチゴのそれらに類似していた．したがって，アイノコキイチゴはナワシロイチゴとエビガライチゴの雑種であるとする見解を支持する結果を得ることができた．またアイノコキイチゴのほとんどの花粉母細胞は第一中期において7個の二価染色体をもつことから，ナワシロイチゴとエビガライチゴは類似のゲノムをもつ近縁種であることが示唆された．

（富山大学理学部生物学教室）